

Figure 1: Real-time PCR data using the Taqman probe

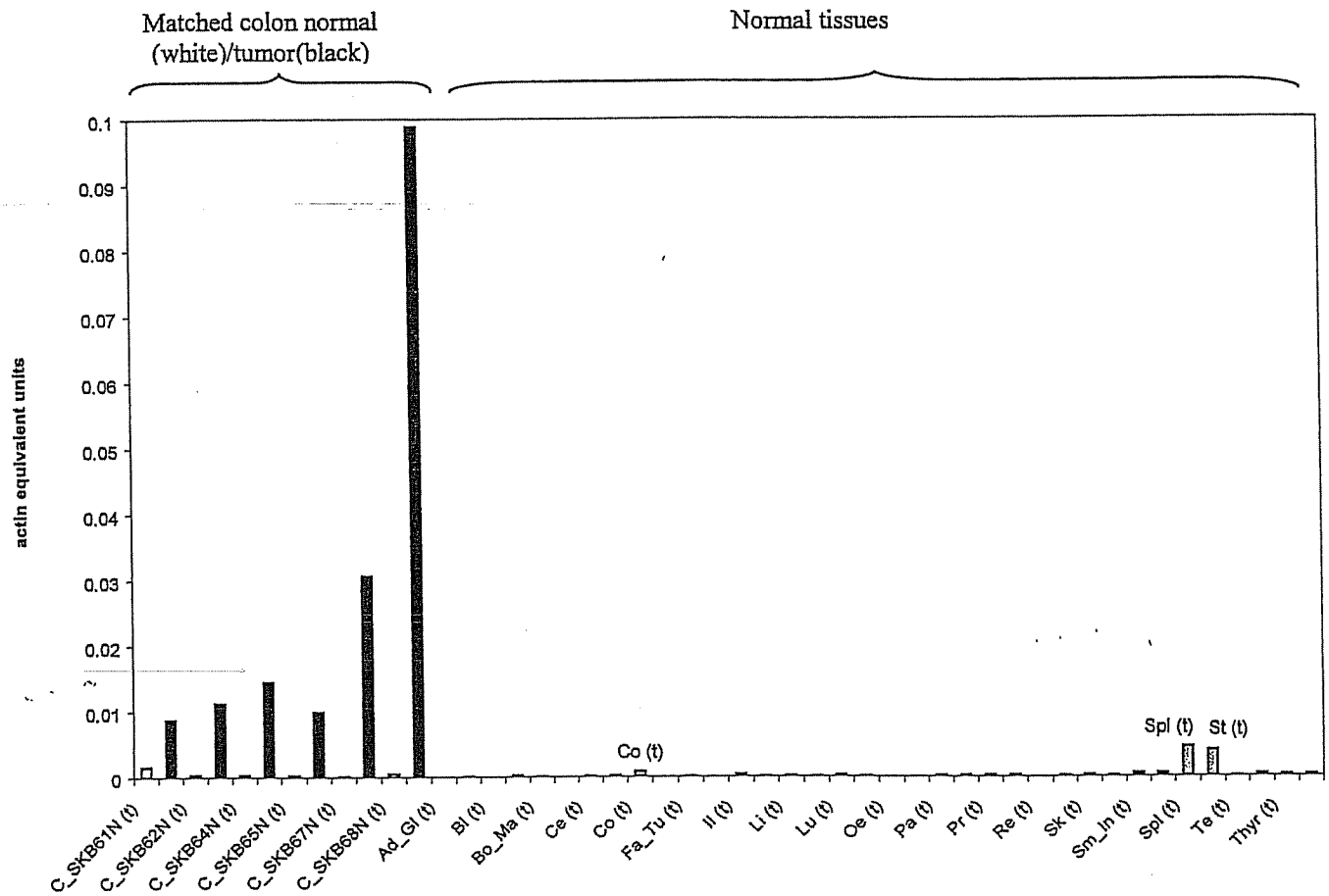


Figure 2: Real-time PCR expression using Sybr protocol

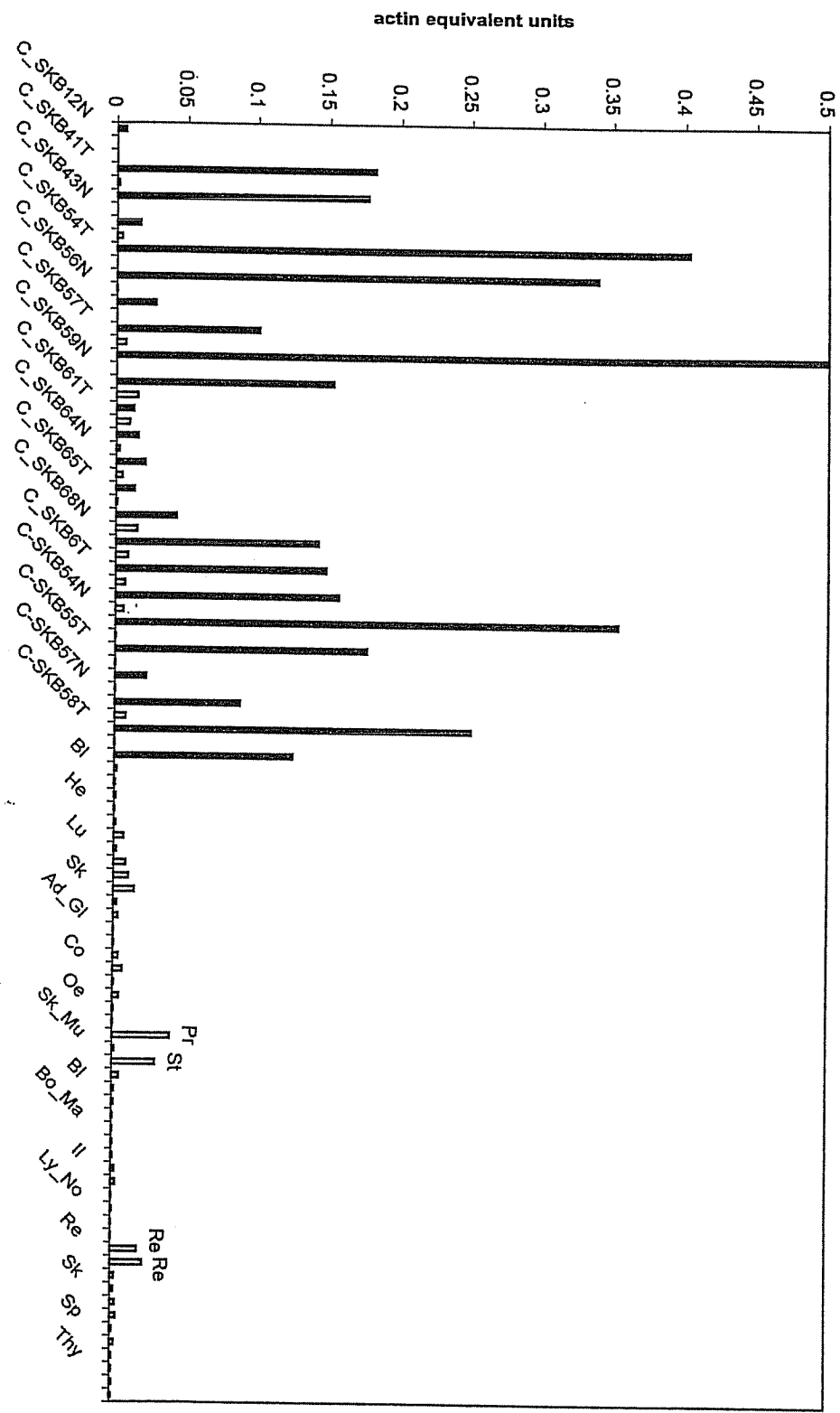


Figure 3: Coomassie blue stained SDS PAGE of the cell extract from the strain expressing CASB7439

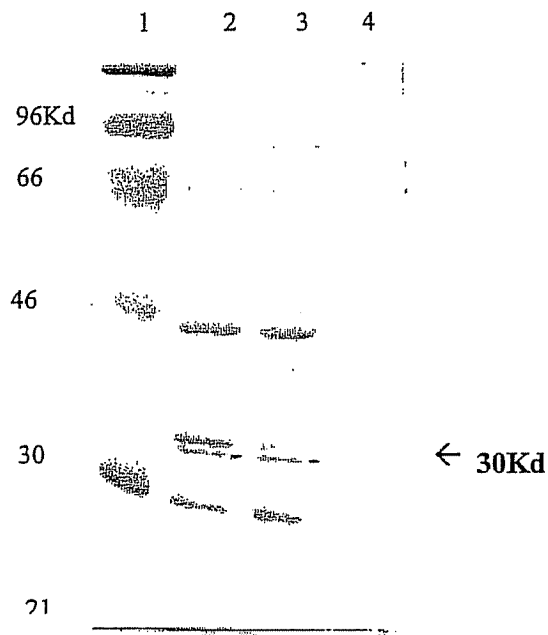


Figure 4: Western blot loaded with cell extract from the strain expressing CASB7439

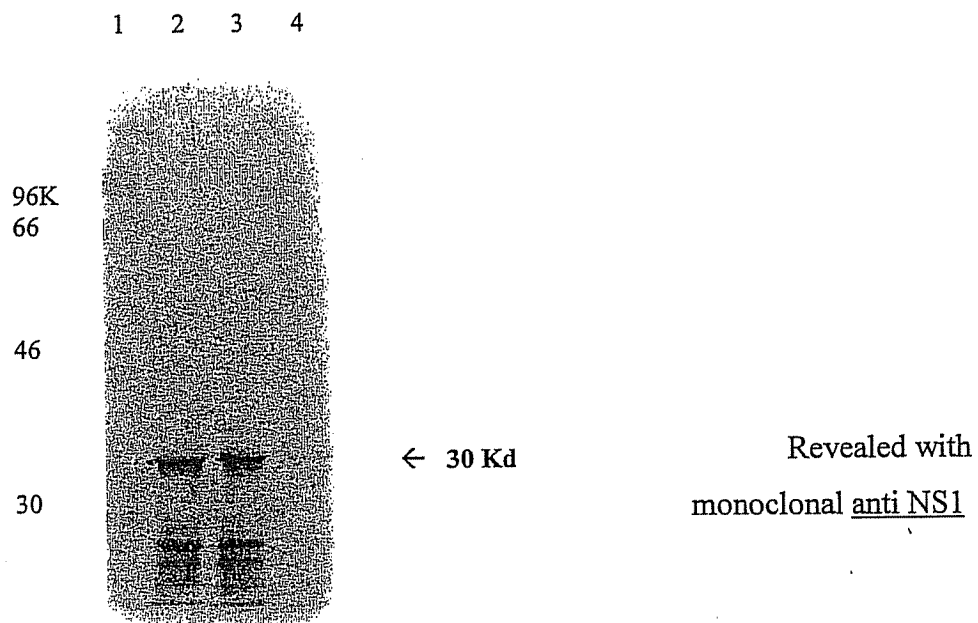
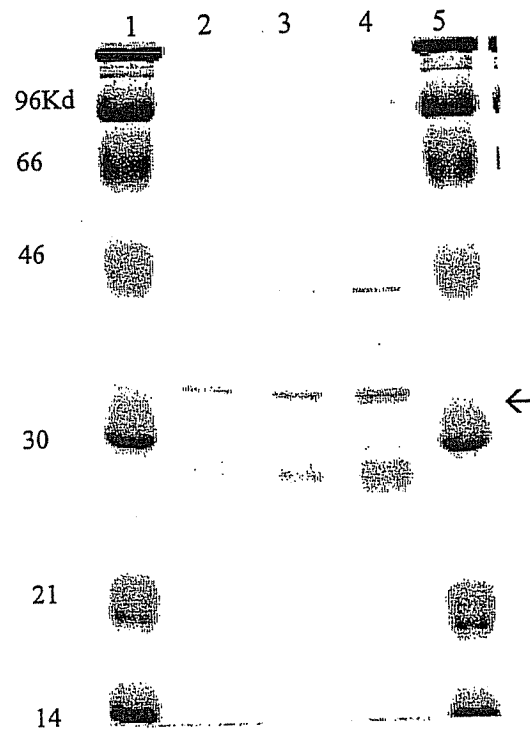
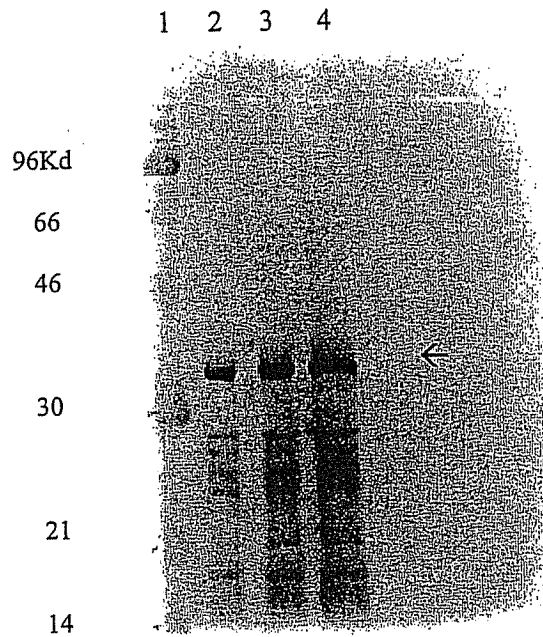


Figure 5: Coomassie-blue stained SDS-PAGE of CASB7439 after purification



6/35

Figure 6: Western blot CASB7439 after purification (monoclonal anti-polyhistidine)



7/35

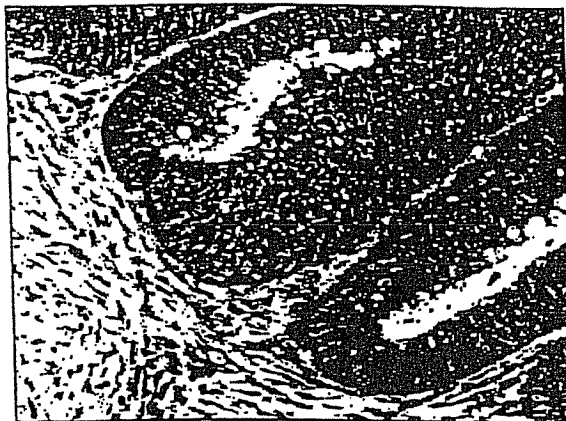


Figure 7  
CASB7439 IHC on a Colon Tumour Biopsy

8/35

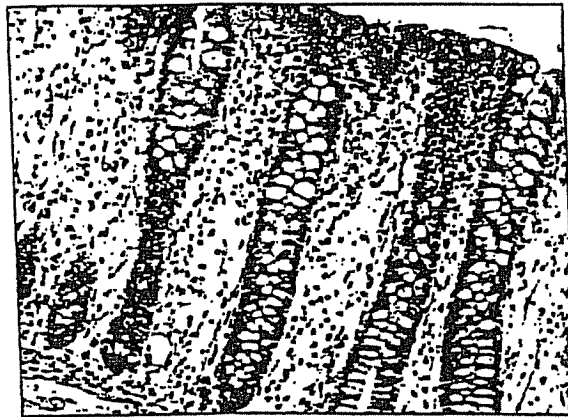
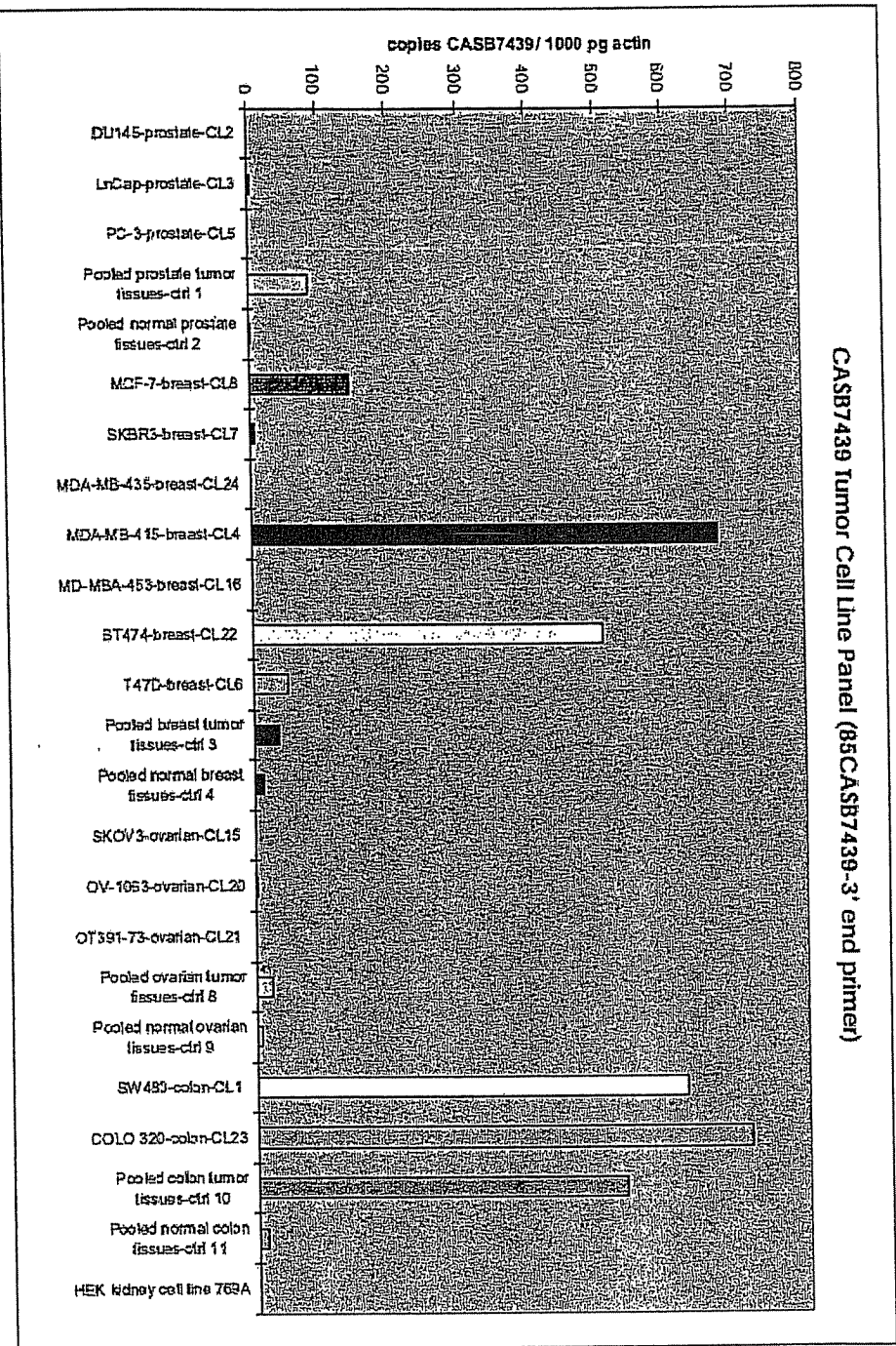


Figure 8  
CASB7439 IHC on a Colon Normal Mucosa Biopsy



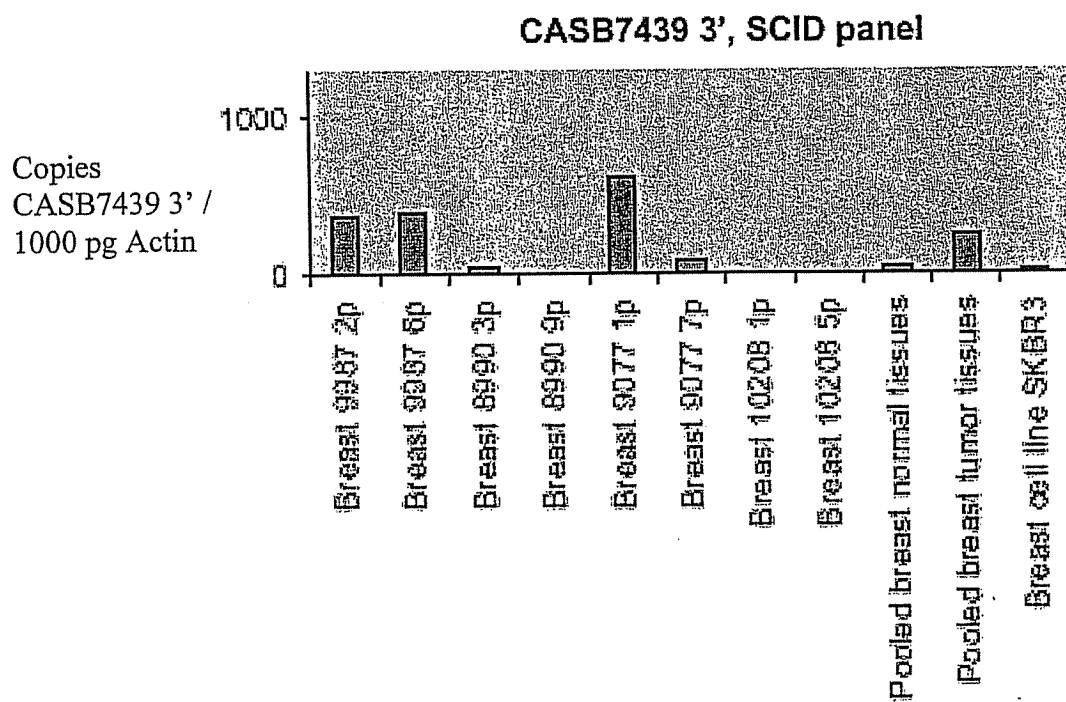
9/35

Figure 9: Real-time RT-PCR quantification of CASB7439 transcript on a panel of cell lines, tumor and normal tissues.



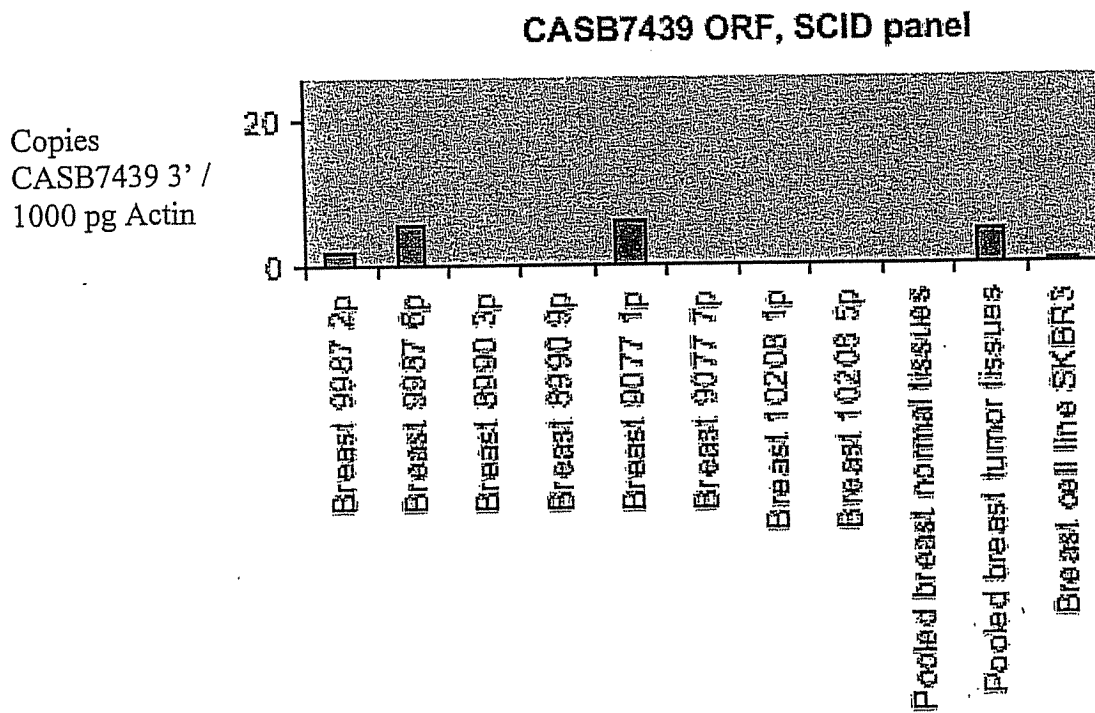
10/35

Figure 10: Real-time RT-PCR quantification (3' UTR primers) of CASB7439 transcript on a panel of tumours grown in SCID mice.



11/35

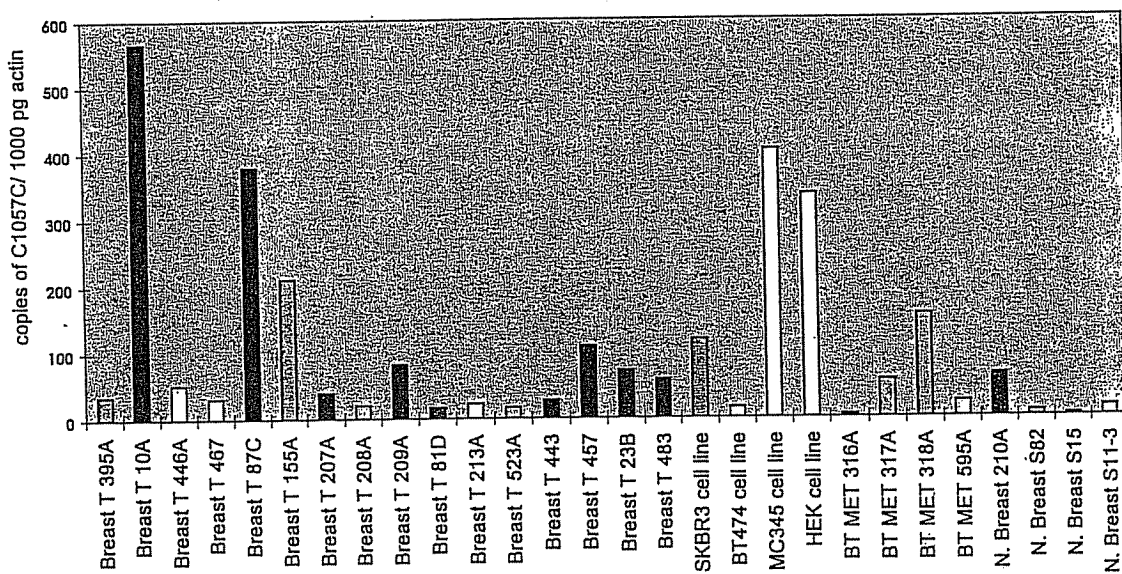
Figure 11: Real-time RT-PCR quantification (ORF primers) of CASB7439 transcript on a panel of tumours grown in SCID mice.



12/35

Figure 12: Real-time RT-PCR quantification (3' UTR primers) of CASB7439 transcript on an extended panel of normal and tumours tissues.

### CASB7439 3', Extended Breast Panel



13/35

Figure 13: Real-time RT-PCR quantification (3' UTR primers) of CASB7439 transcript on an extended panel of normal and tumours tissues.

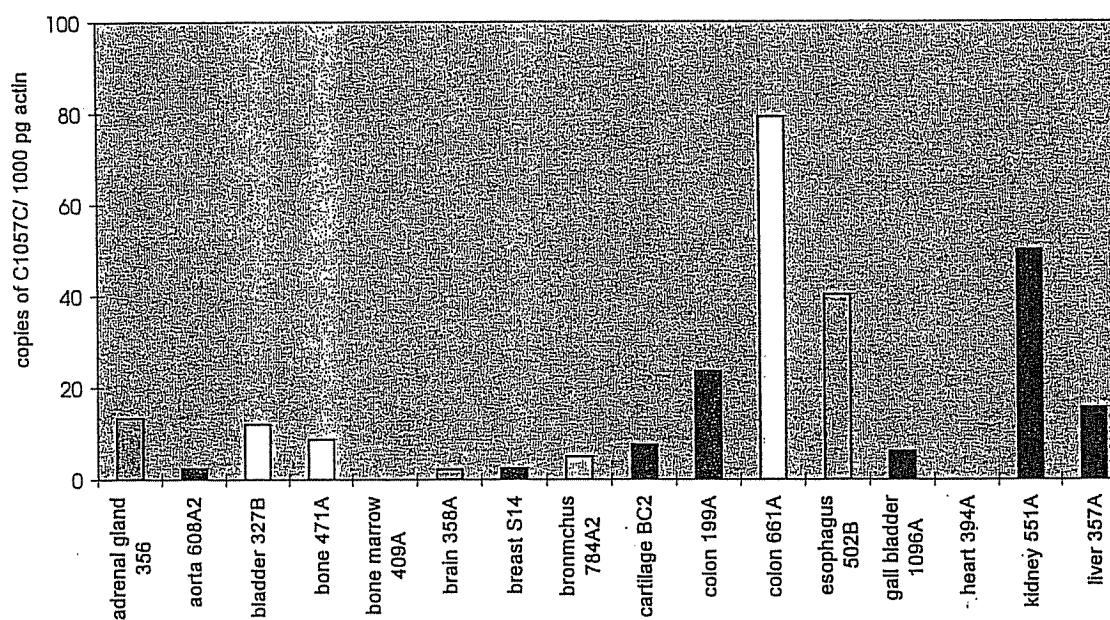


Figure 14: Real-time RT-PCR quantification (3' UTR primers) of CASB7439 transcript on an extended panel of normal and tumours tissues, continued.

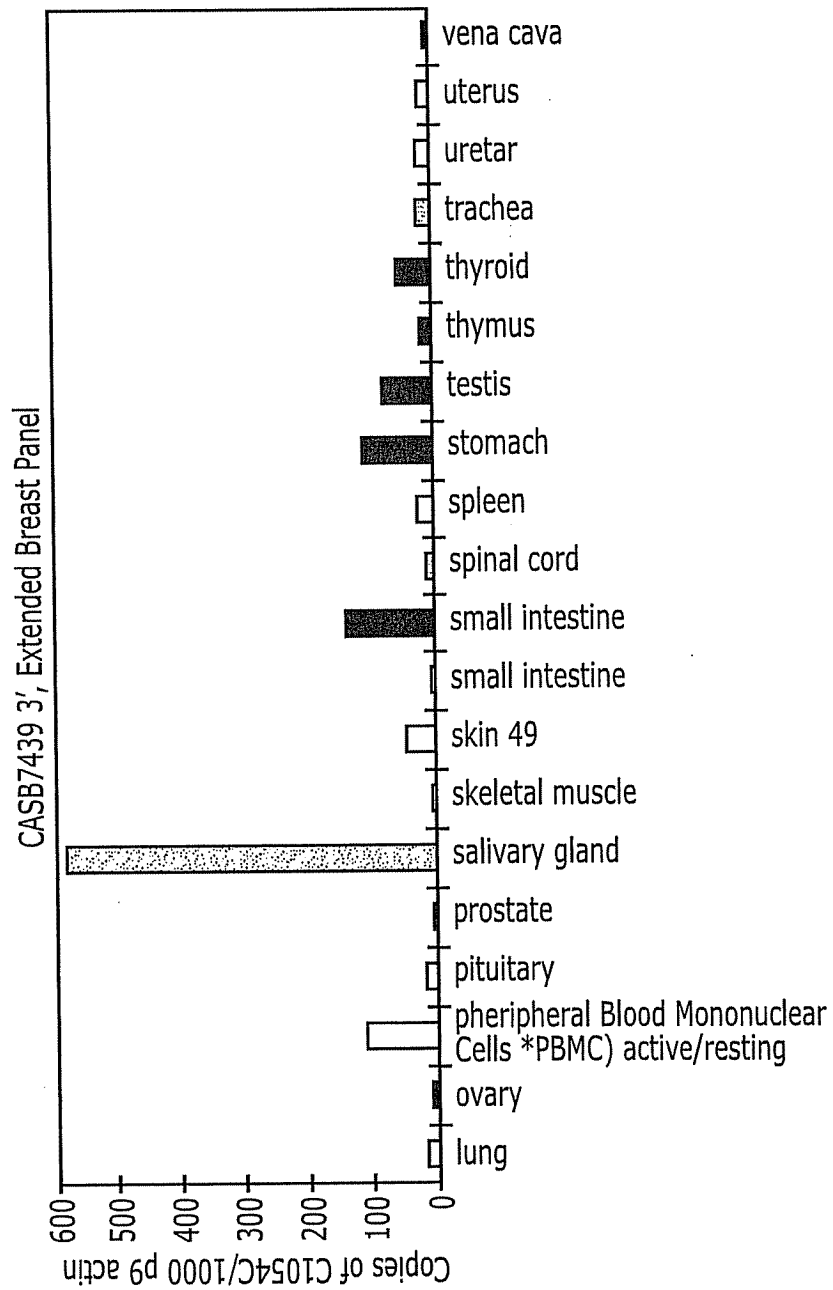


Figure 15: Design of NS1-CASB7439 protein.

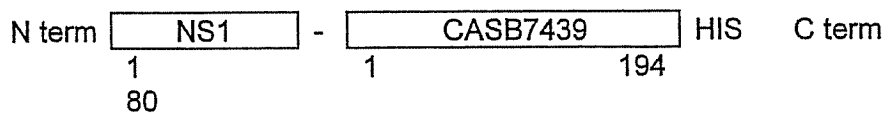


Figure 16: Schematic representation of pMG1 cloning strategy (constructs 1 and 2).

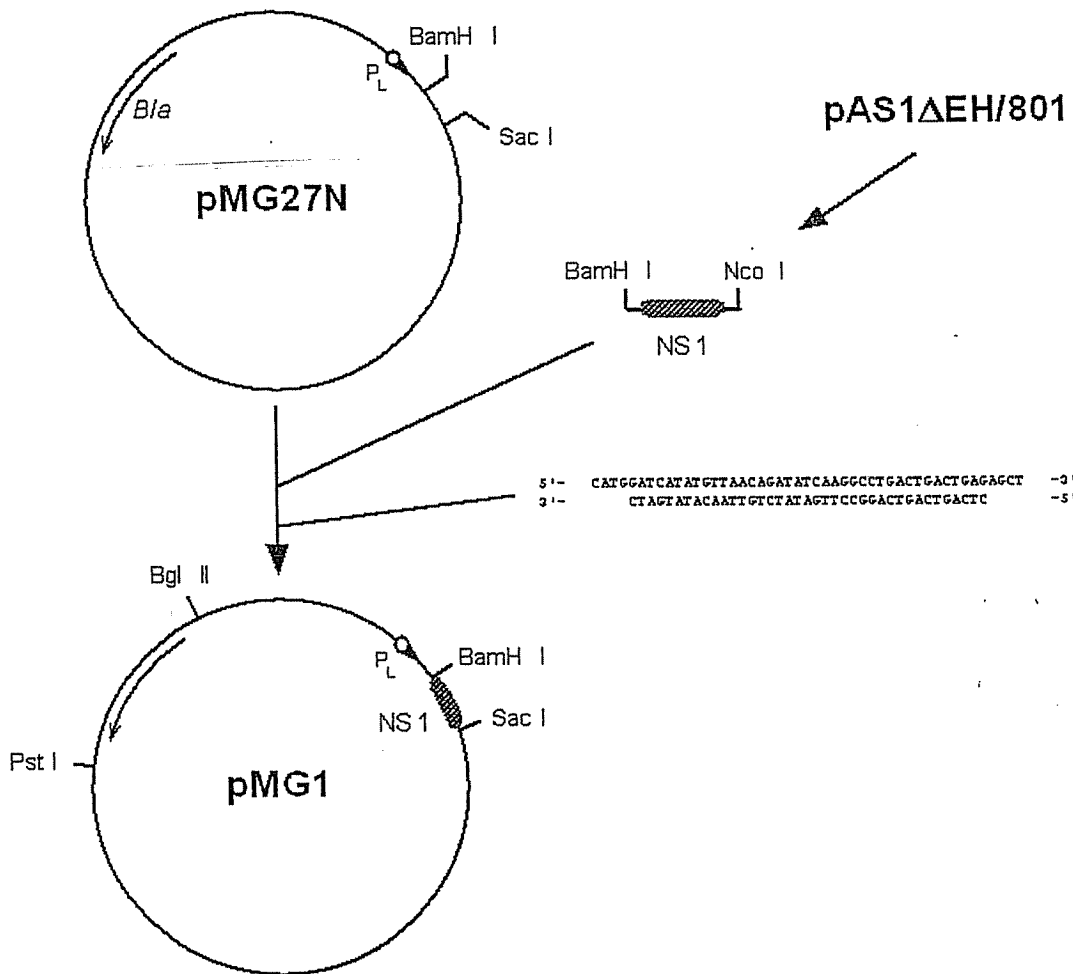
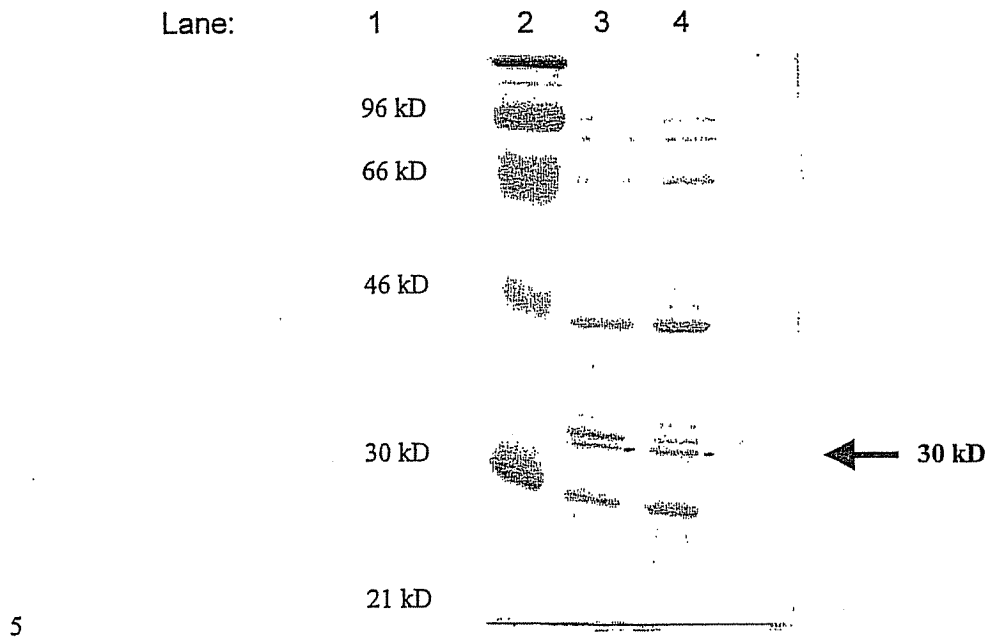




Figure 17: Coomassie blue stained SDS PAGE of the cell extract from the strain expressing CASB7439 construct 1.



Legend:

Lane 1: Molecular weights

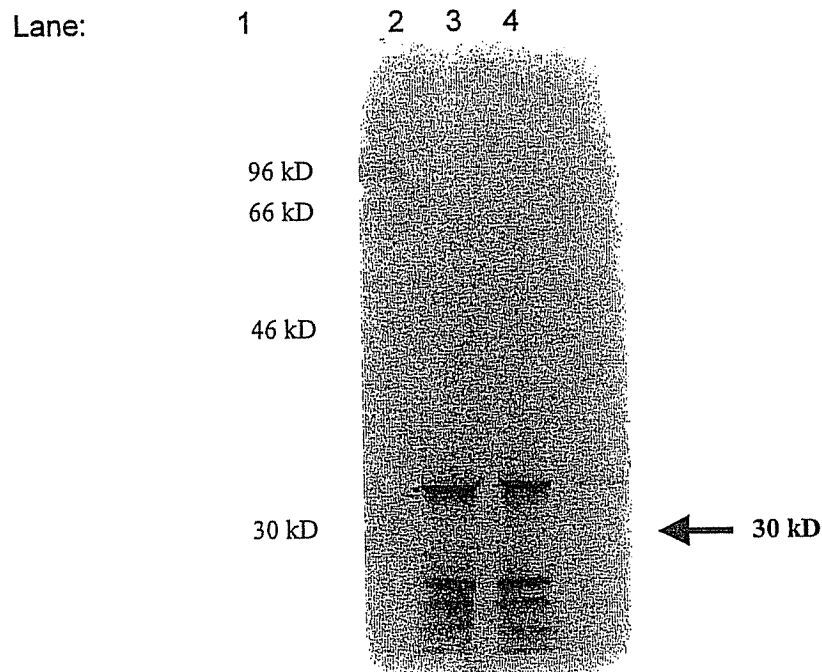
Lane 2: Whole cellular extract

10 Lane 3: Supernatant of cellular extract

Lane 4: Pellet of cellular extract

18/35

Figure 18: Western blot loaded with cell extract from the strain expressing CASB7439 construct 1.



**Legend:**

Lane 1: Molecular weights

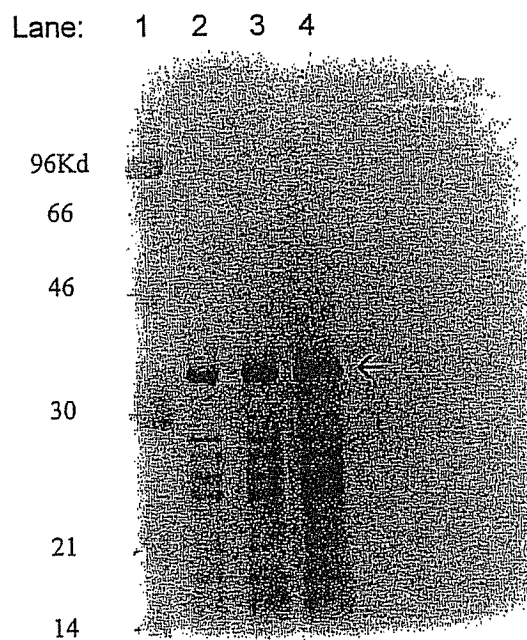
Lane 2: Whole cellular extract

Lane 3: Supernatant of cellular extract

Lane 4: Pellet of cellular extract

19/35

Figure 19: Western blot CASB7439 construct 1 after purification (monoclonal anti-NS1 N terminal fragment).



Legend:

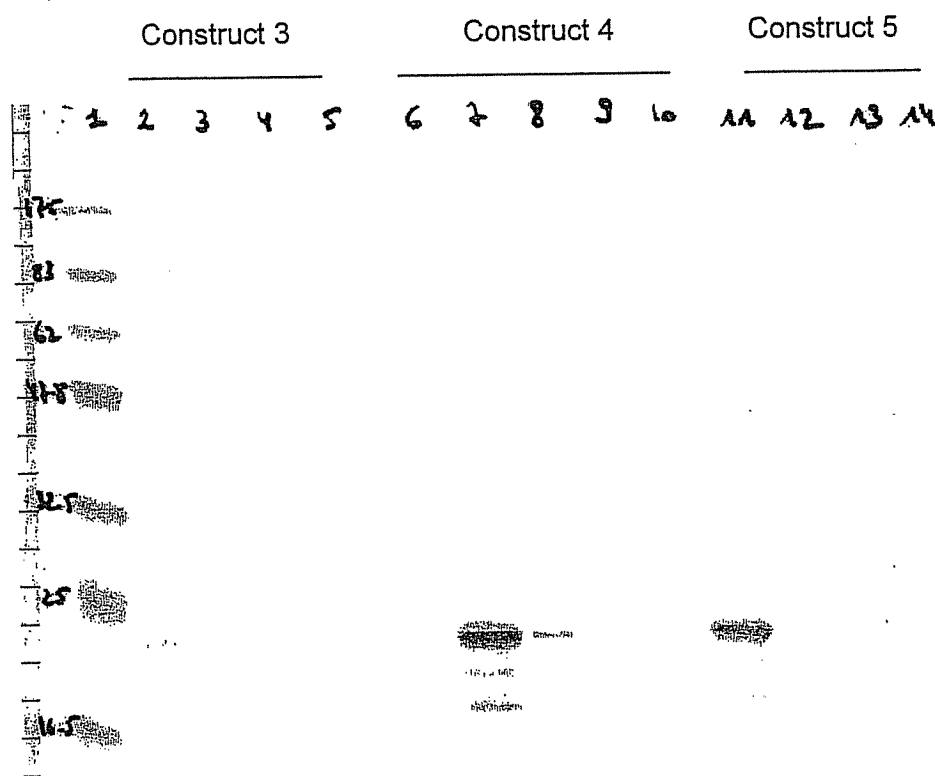
Lane 1: Molecular weights

Lane 2: 2  $\mu$ l of purified protein

Lane 3: 4  $\mu$ l of purified protein

Lane 4: 6  $\mu$ l of purified protein

Figure 20: Comparison of Constructs 3 to 5 expression yields. Western blot on CASB7439 constructs 3 to 5 expressed in *E. coli*, revealed with anti-peptide SB600 antibody.

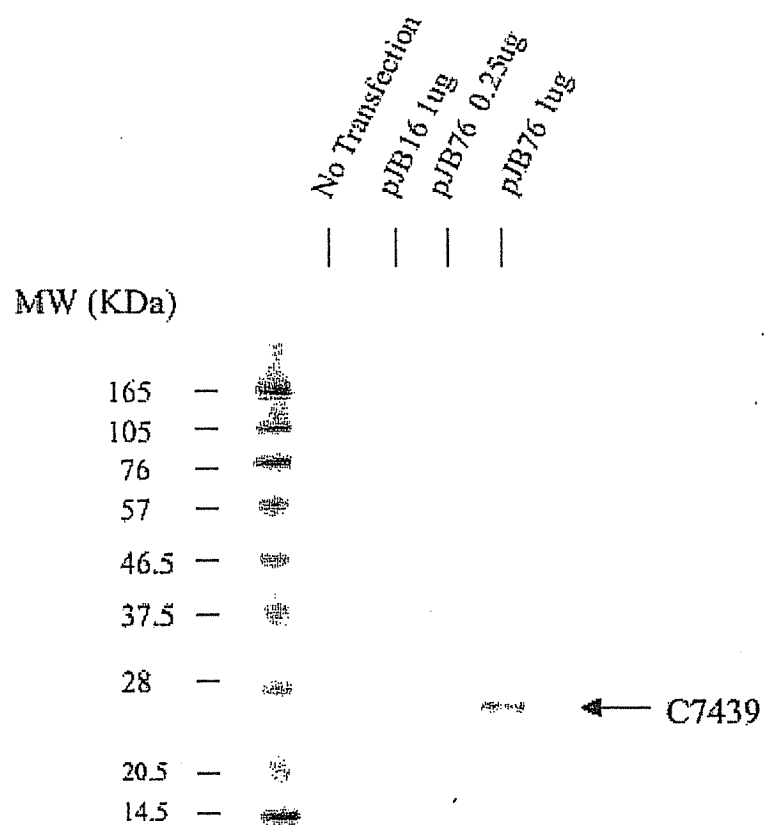


Legend:

- Lane 1: MW; Kd
- Lane 2: Construct 2, undiluted.
- Lane 3: Construct 2, 1/10 dilution.
- Lane 4: Construct 2, 1/50 dilution.
- Lane 5: Construct 2, 1/100 dilution.
- Lane 6: Construct 3, undiluted.
- Lane 7: Construct 3, undiluted.
- Lane 8: Construct 3, 1/10 dilution.
- Lane 9: Construct 3, 1/50 dilution.
- Lane 10: Construct 3, 1/100 dilution.
- Lane 11: Construct 4, undiluted.
- Lane 12: Construct 4, 1/10 dilution.
- Lane 13: Construct 4, 1/50 dilution.
- Lane 14: Construct 4, 1/100 dilution.

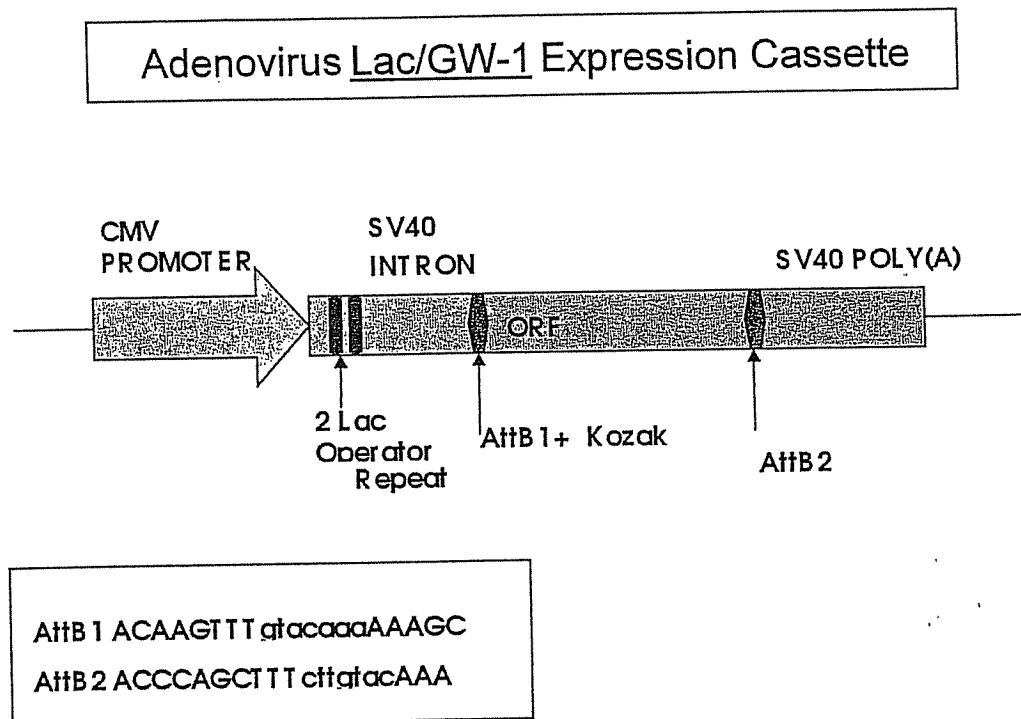
Figure 21: Expression of C7439 in HEK 293T Cells by DNA Vaccine Vector pJB76 (pJB16/C7439)

# Expression of C7439 in HEK 293T Cells by DNA Vaccine Vector pJB76 (pJB16/C7439)



22/35

Figure 22: Schematic representation of CASB7439 expressed in an adenoviral live vector



23/35

Figure 23: Expression of C7439 in Human Fibroblasts by Recombinant Adenovirus AdC7439

## Expression of C7439 in Human Fibroblasts by the Recombinant Adenovirus AdC7439

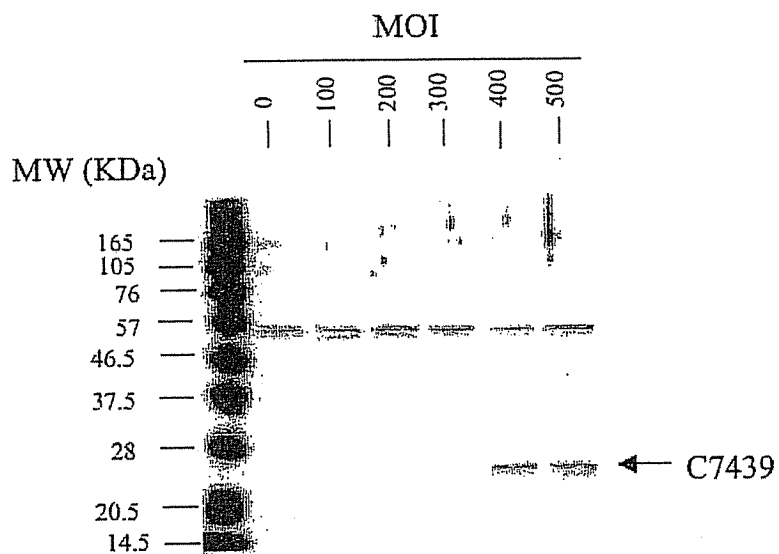


Figure 24: Real-time RT-PCR quantification of CASB7439 transcript on a population of preneoplastic lesions of lung (PLL) patients.

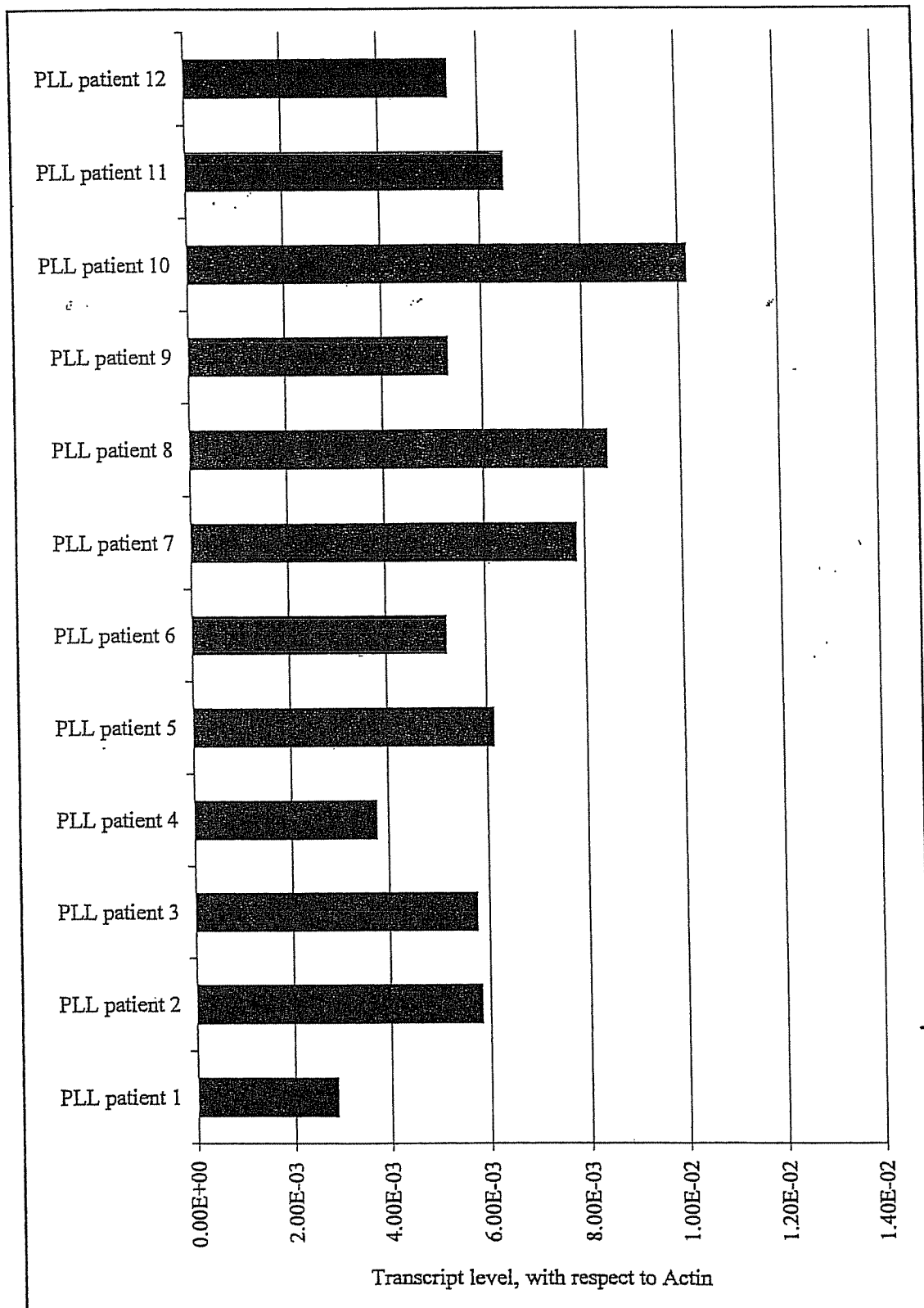




Figure 25: Real-time RT-PCR quantification of CASB7439 transcript on a population of early stage lung cancer (ESLC) patients.

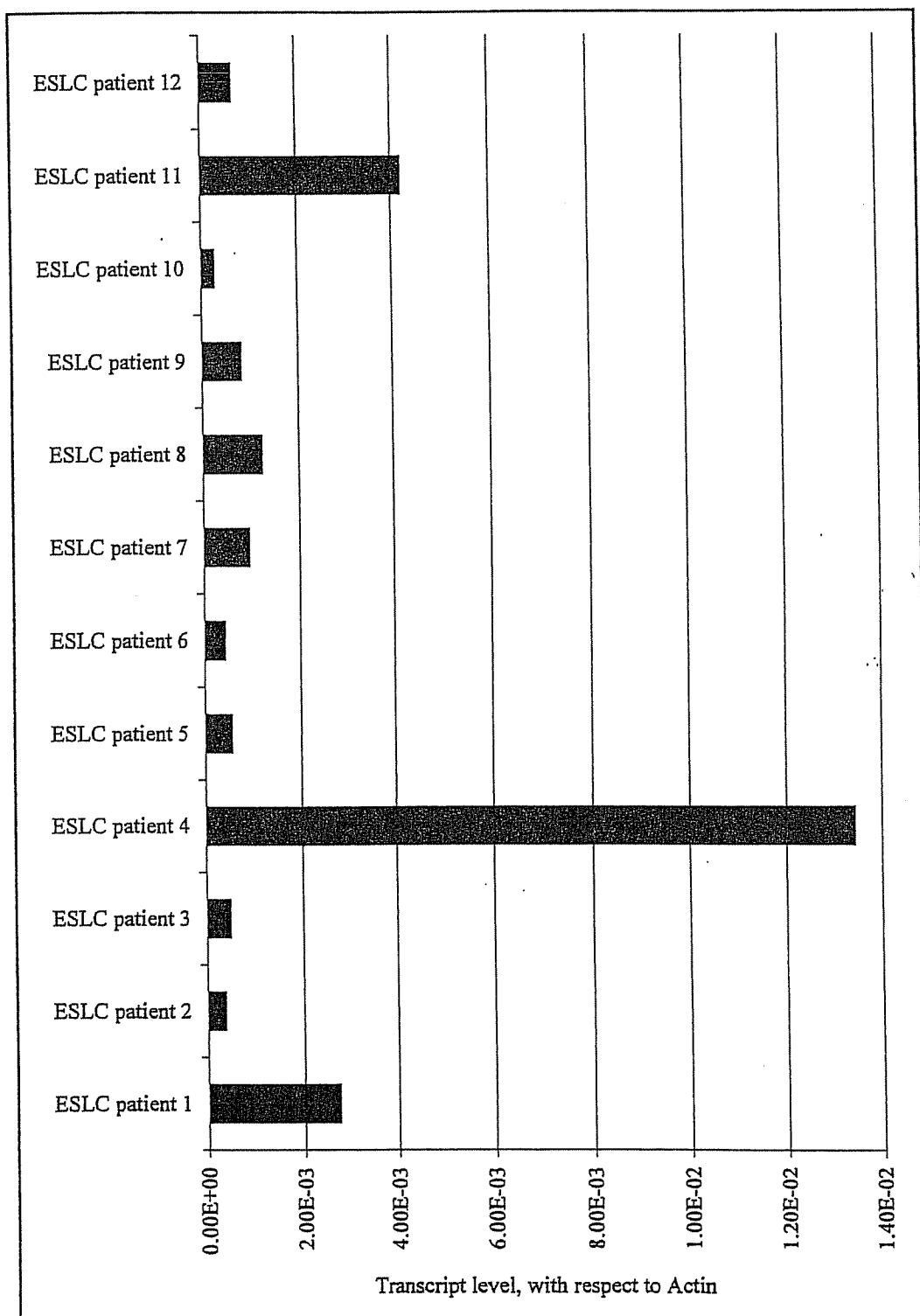
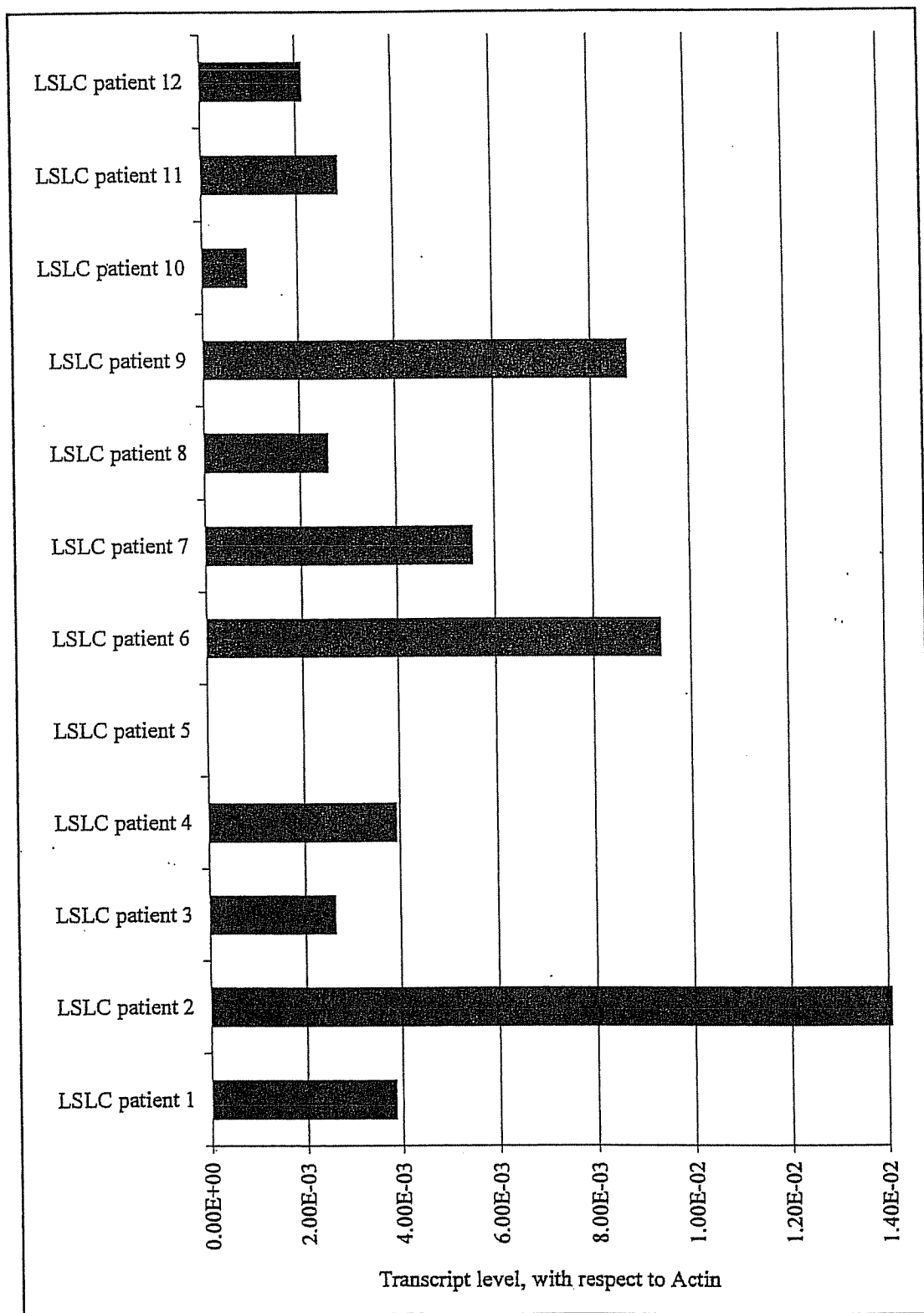


Figure 26: Real-time RT-PCR quantification of CASB7439 transcript on a population of late stage lung cancer (LSLC) patients.



27/35

Figure 27: Real-time RT-PCR quantification of CASB7439 transcript on a panel of normal tissues.

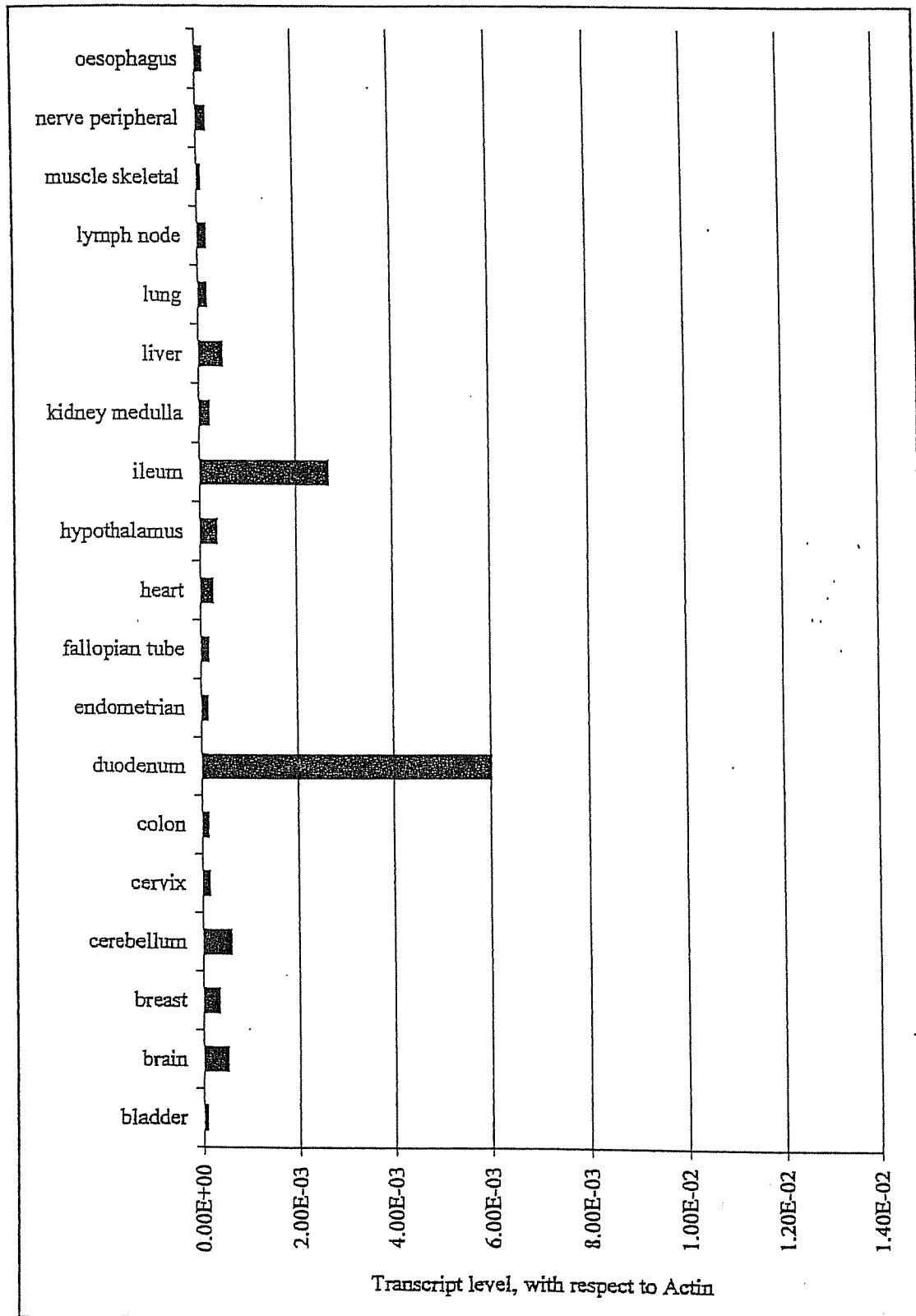


Figure 28: Real-time RT-PCR quantification of CASB7439 transcript on a panel of normal tissues, continued.

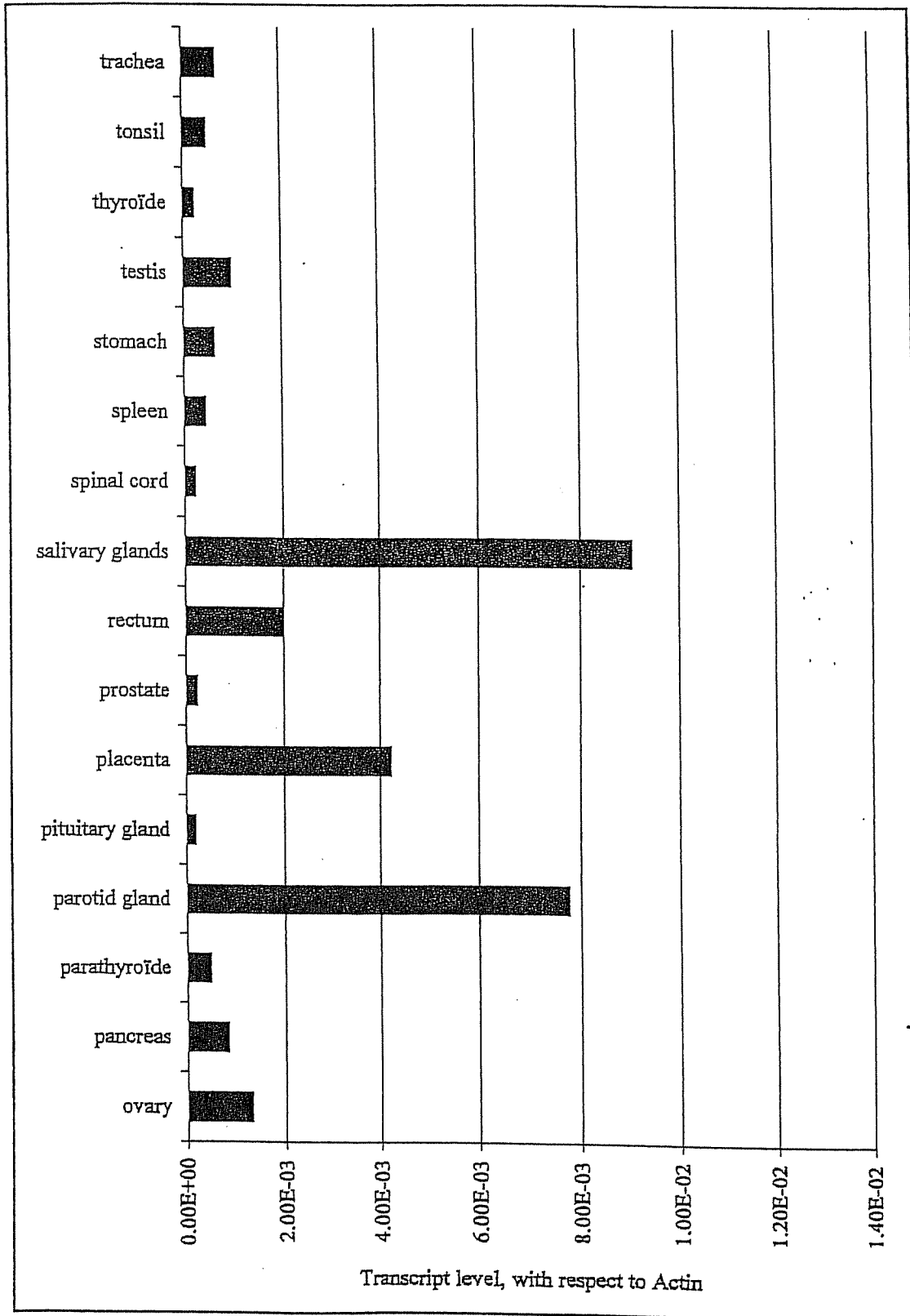


Figure 29: Design of NS1-CASB7439 protein.

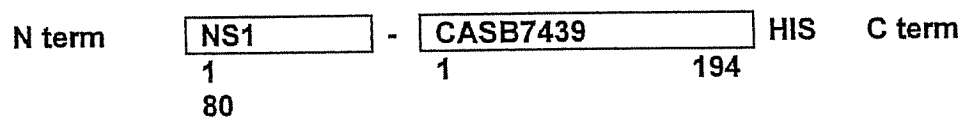
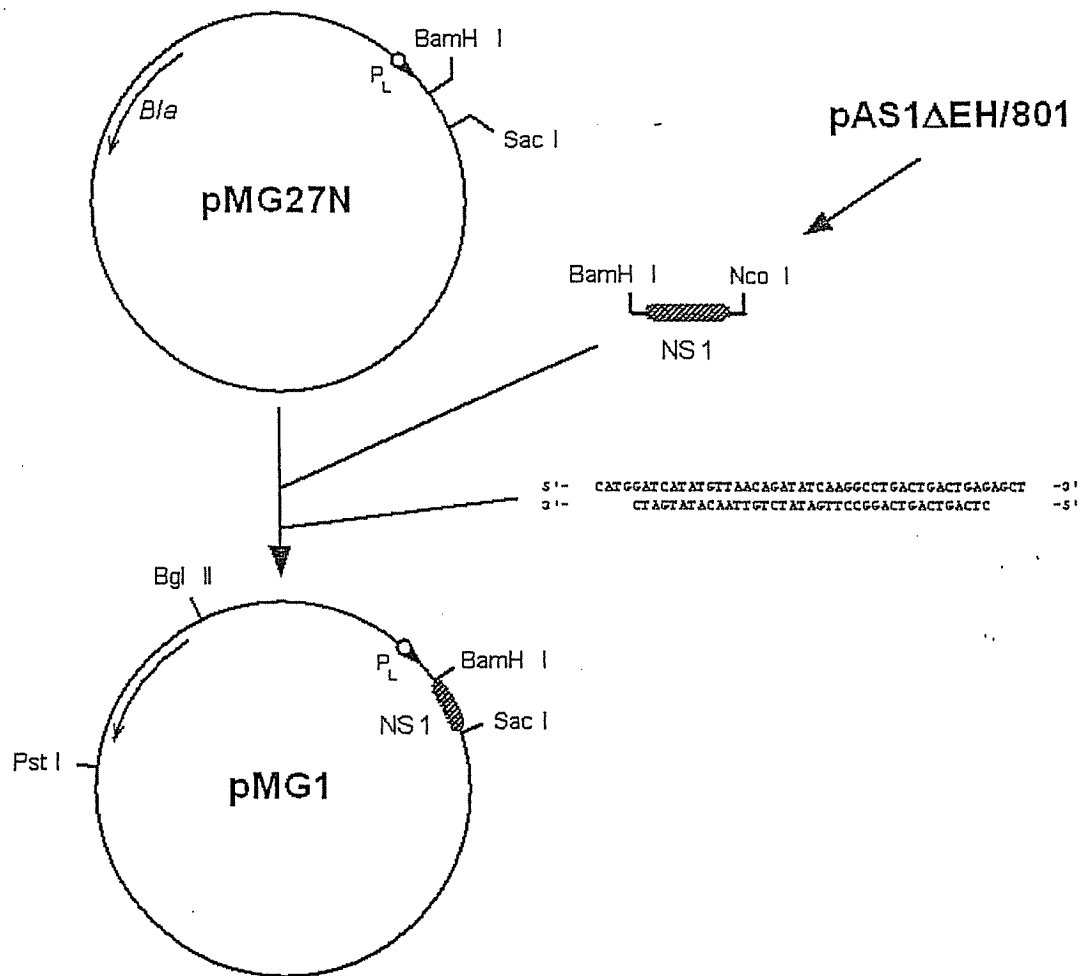
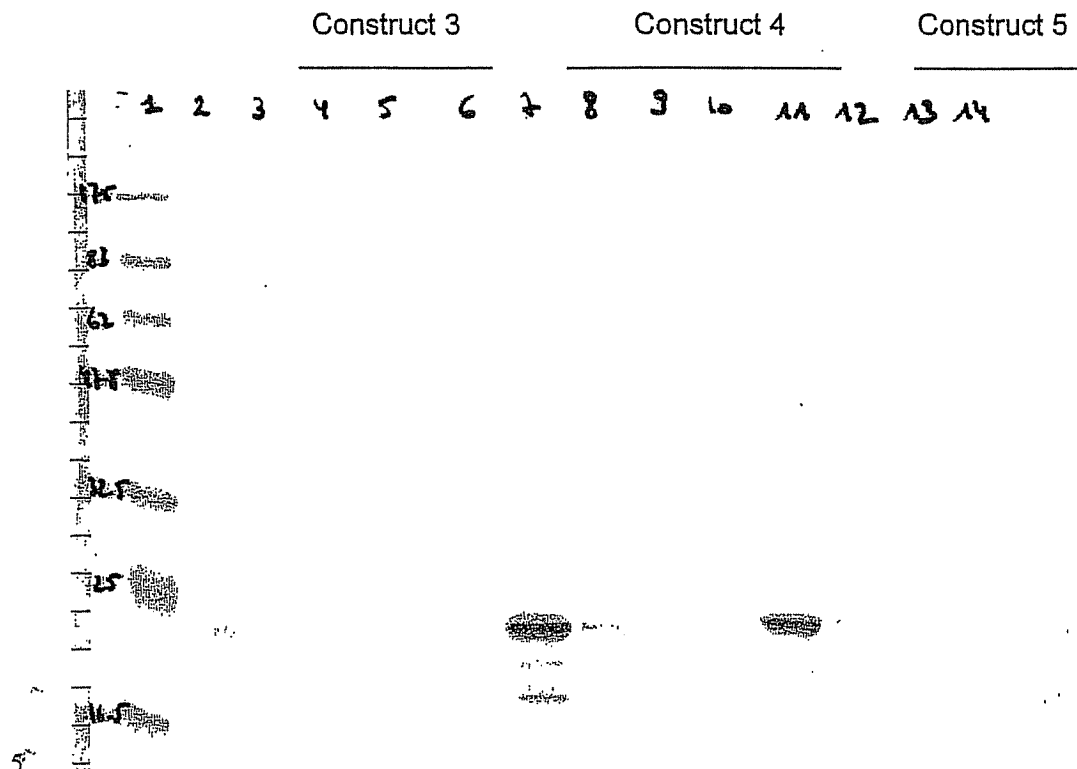


Figure 30: Schematic representation of pMG1 cloning strategy (constructs 1 and 2).



31/35

Figure 31: Comparison of Constructs 3 to 5 expression yields. Western blot on CASB7439 constructs 3 to 5 expressed in *E. coli*, revealed with anti-peptide SB600 antibody.

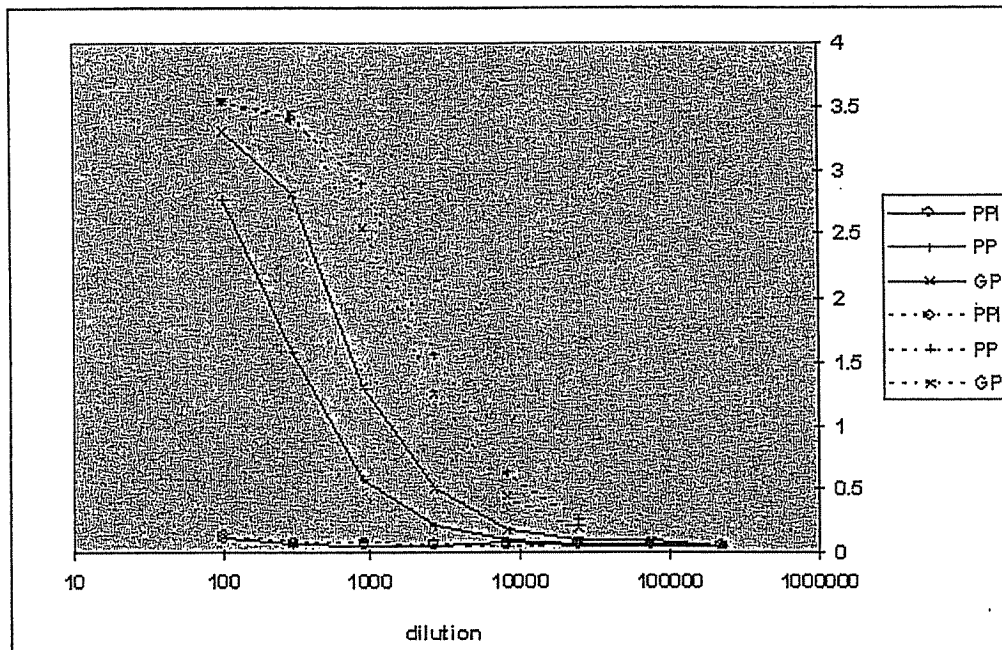


Legend:

- Lane 1: MW; Kd
- Lane 2: Construct 2, undiluted.
- Lane 3: Construct 2, 1/10 dilution.
- Lane 4: Construct 2, 1/50 dilution.
- Lane 5: Construct 2, 1/100 dilution.
- Lane 6: Construct 3, undiluted.
- Lane 7: Construct 3, undiluted.
- Lane 8: Construct 3, 1/10 dilution.
- Lane 9: Construct 3, 1/50 dilution.
- Lane 10: Construct 3, 1/100 dilution.
- Lane 11: Construct 4, undiluted.
- Lane 12: Construct 4, 1/10 dilution.
- Lane 13: Construct 4, 1/50 dilution.
- Lane 14: Construct 4, 1/100 dilution.

32/35

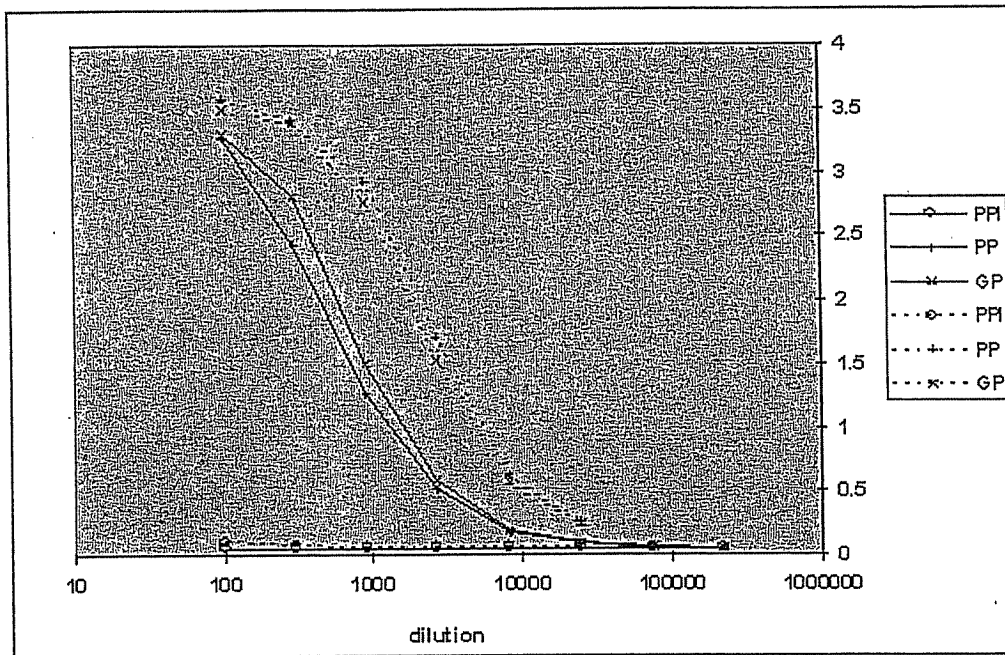
Figure 32: CASB7439 peptide 1 immunization of rabbit SB598: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 1, straight line; antibody titers relative to KLH carrier, discontinuous line)





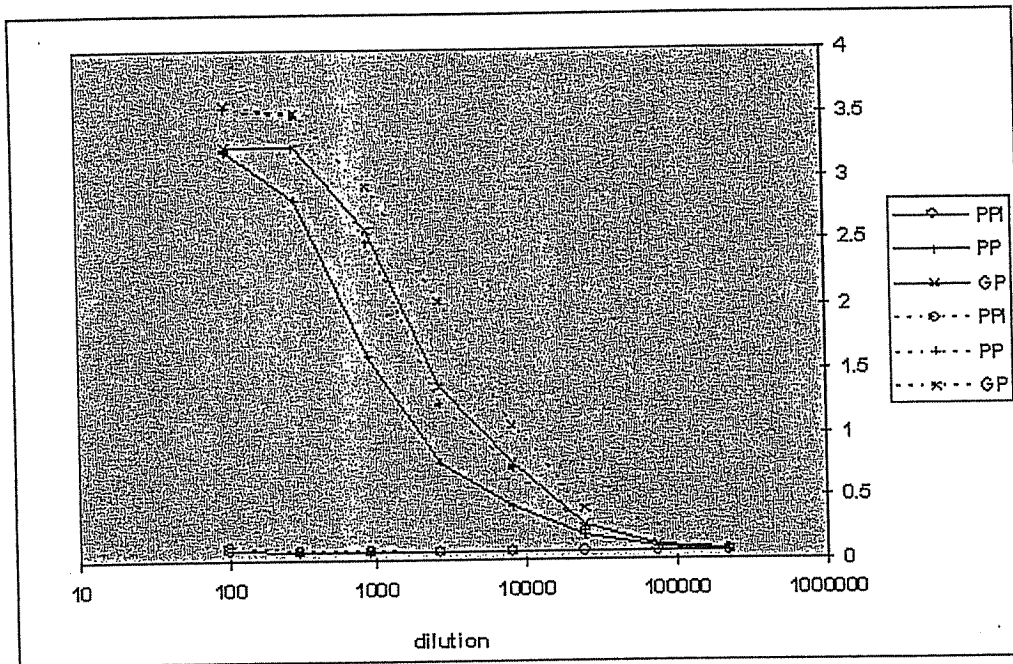
33/35

Figure 33: CASB7439 peptide 1 immunization of rabbit SB599: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 1, straight line; antibody titers relative to KLH carrier, discontinuous line).



5.

Figure 34: CASB7439 peptide 2 immunization of rabbit SB600: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 2, straight line; antibody titers relative to KLH carrier, discontinuous line).



35/35

Figure 35: CASB7439 peptide 2 immunization of rabbit SB601: graphical view of ELISA read-outs (Antibody titers relative to CASB7439 peptide 2, straight line; antibody titers relative to KLH carrier, discontinuous line).

